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REVIEW

Biomarker discovery for renal cancer stem cells

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Abstract

Characterised by high intra- and inter-tumor heterogeneity, metastatic renal cell carcinoma (RCC) is resistant to chemo- and radiotherapy. Therefore, the development of new prognostic and diagnostic markers for RCC patients is needed. Cancer stem cells (CSCs) are a small population of neoplastic cells within a tumor which present characteristics reminiscent of normal stem cells. CSCs are characterised by unlimited cell division, maintenance of the stem cell pool (self-renewal), and capability to give rise to all cell types within a tumor; and contribute to metastasis *in vivo* (tumourigenicity), treatment resistance and recurrence. So far, many studies have tried to establish unique biomarkers to identify CSC populations in RCC. At the same time, different approaches have been developed with the aim to isolate CSCs. Consequently, several markers were found to be specifically expressed in CSCs and cancer stem-like cells derived from RCC such as CD105, ALDH1, OCT4, CD133, and CXCR4. However, the contribution of genetic and epigenetic mechanisms, and tumor microenvironment, to cellular plasticity have made the discovery of unique biomarkers a very difficult task. In fact, contrasting results regarding the applicability of such markers to the isolation of renal CSCs have been reported in the literature. Therefore, a better understanding of the mechanism underlying CSC may help dissecting tumor heterogeneity and drug treatment efficiency.

Keywords: cancer stem cells; tumor-initiating cells; renal cell carcinoma; biomarkers

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Introduction

Renal cell carcinoma (RCC), a malignant tumor affecting the adult kidney, accounts for 2% of all cancers. It affects 64 000 people every year, with a mortality of 20%, and is among the 10 most common cancers worldwide [1]. Arising from renal tubular epithelial cells, RCC is the most frequent malignancy affecting the adult kidney (87%) [2].

RCC is a very heterogeneous class of tumors [3]. According to the classification proposed by the World Health Organization in 2016, which combines histological and genetic characteristics and clinical implications, RCC can be subdivided into three different entities [3,4]. Clear cell renal cell carcinoma (ccRCC) is the most common subtype of RCC and represents up to 80% of all RCCs [5,6]. Papillary renal cell carcinoma (pRCC) accounts for 10–15% of all RCCs [7–9], whereas chromophobe RCC makes up only the remaining 5% [10].

RCC is characterised by being asymptomatic at early stage and showing a poor response to radiotherapy and chemotherapy once metastatic, making this tumor type very difficult to diagnose and treat [11]. Due to the much higher prevalence of ccRCCs, very few clinical trials have been carried out considering other histological RCC subtypes. Therefore, most of the drugs have been developed based on ccRCC, but they are currently applied to all RCC patients. Treatment of advanced or metastatic RCC patients is achieved primarily by targeted therapies [tyrosine kinase inhibitors (TKIs)] and 5-year survival for these patients is 12% [12]. Despite all the progress made in the development of novel anticancer compounds, the management and treatment of RCC patients still remains a crucial aspect in the clinic.

In particular, intra-tumor and inter-tumor heterogeneity is one of the major limitations in the treatment of epithelial tumors [13]. Two different tumor models were proposed to play a role in tumor development, progression, and tumor heterogeneity. The clonal evolution model or stochastic model implies the presence of a tumor cell population carrying different mutations which have accumulated over time and then selected

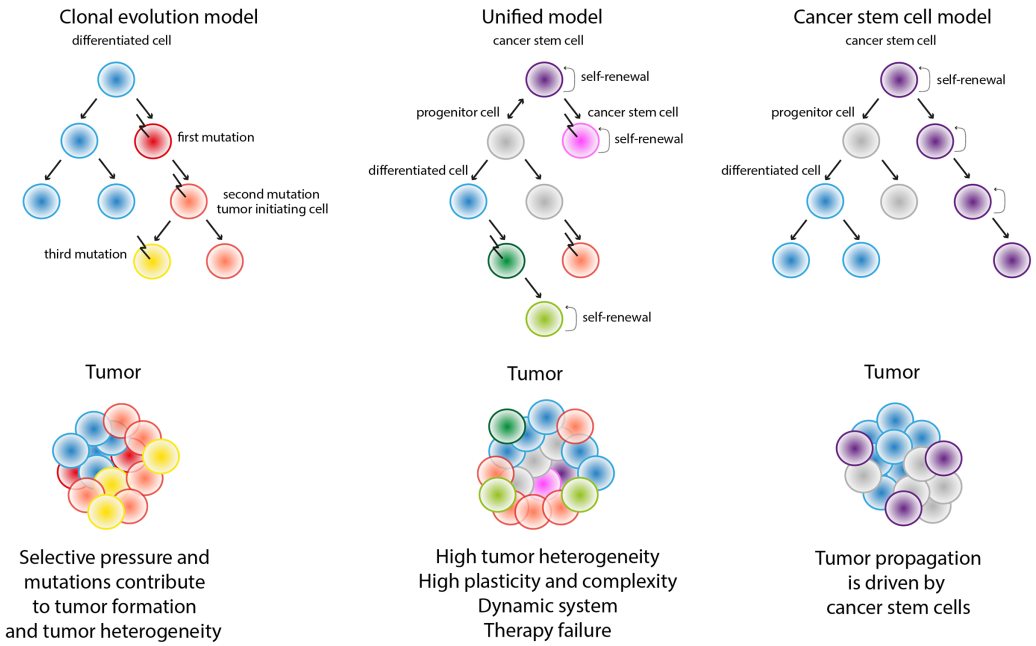

Figure 1. Models of tumourigenesis. This figure illustrates three models of tumourigenesis. The clonal evolution model or stochastic model (left) implies the presence of a tumor cell population carrying multiple mutations which is transformed over time by selective pressure, resulting in tumor heterogeneity and progression. The CSC model or hierarchical model (right) proposes that tumor growth and propagation are driven by a small subpopulation of cells with pluriproliferative features, namely CSCs. More recently, a unifying model (centre) characterised by high tumor heterogeneity, plasticity, and complexity has been proposed. According to this model, CSCs can acquire mutations and generate new stem cell branches. Conversely, tumor cells in the non-CSC subpopulation can undergo EMT and acquire CSC-like features, contributing to tumor heterogeneity. Moreover, TME and therapy add another layer of complexity.

under different selective pressures [14]. Every cell within a tumor has potentially the same likelihood to facilitate tumor formation and progression. Selection, clonal expansion and genetic instability are the key elements driving the stochastic approach [15]. The cancer stem cell (CSC) model, or hierarchical model, instead, proposes that tumor growth and propagation is driven by a small phenotypically distinct subset of cells with pluriproliferative features within the total cancer cell population [16,17]. According to this model, the tumor bulk is established by a pool of CSCs that have both stem cell potential and the ability to give rise to progeny with self-limited proliferative capacity [18]. As a result of this model, elimination of the entire CSC population will result in tumor eradication, whereas leaving even only one cell behind will lead to tumor recurrence [15,19].

Nevertheless, it is becoming increasingly clear that genetic and epigenetic factors are not the only two factors contributing to tumor heterogeneity. The tumor microenvironment (TME), stromal cells, soluble molecules, and extracellular vesicles (i.e. exosomes) play an important role in modulating metastatic properties and sensitivity of tumor cells to therapy [20,21]. Therapy itself may act as selection mechanism that shapes tumor evolution. More recently, a unifying model of clonal evolution applied to CSCs was proposed by Kreso *et al*, whereby CSCs can acquire mutations and generate new stem cell branches and, at the same time, tumor cells in the non-CSC subpopulation can undergo epithelial-mesenchymal transition (EMT) and acquire CSC-like features contributing to tumor heterogeneity [17] (Figure 1). Processes such as inflammation, hypoxia, angiogenesis, and EMT occurring in the TME contribute to the maintenance of CSC fate. Due to cellular plasticity, it is important to note that the cell of origin – the normal cell that acquires the first genetic hit(s) that culminate in the initiation of cancer – does not necessarily refer to the CSC population as the hierarchical model would suggest. CSCs are the cellular subset within the tumor that uniquely sustains malignant growth. Cells-of-origin and CSCs refer to tumor-initiating cells (TICs) and cancer-propagating cells, respectively [22].

CSCs are a small population of neoplastic cells within a tumor presenting characteristics reminiscent of normal stem cells (NSCs). In particular, they are capable of giving rise to all the cell types present in the tumor tissue which they derive from (differentiation). They are characterised by unlimited cell

division and maintenance of the stem cell pool (self-renewal). They can give rise to tumor and contribute to metastasis formation *in vivo* (tumourigenicity). Moreover, CSCs are recognised to be the major cause of tumor recurrence and resistance to therapy.

Dick and co-authors performed the first experimental study on CSCs in 1994. They isolated $CD34^+/CD38^-$ cells from acute myeloid leukemia (AML) patients and showed that they could initiate AML *in vivo* upon transplantation into NOD/SCID mice [23,24]. Subsequently, several others have showed the presence of CSCs in colorectal cancer, breast cancer, glioblastoma, melanoma, lung cancer, liver, and prostate cancer [25–33]. Growing evidence suggests that renal cancer, as many other solid tumors, possesses a rare population of cells capable of self-renewal that contribute to metastasis and resistance to therapy [34]. Therefore, the identification of a specific subpopulation of cells within a tumor that either initiate or maintain tumourigenesis is of utmost importance for understanding tumor biology and in the development of novel therapies. In this review, we outline potential CSC markers in RCC as well as advantages and pitfalls in the identification of these tumor-propagating cells.

Cancer stem cell biomarkers

To date, several markers have been found to be specifically expressed in CSCs and cancer stem-like cells derived from RCC. A summary of these putative CSC markers is given in Table 1.

CD105

CD105 (endoglin) is a transmembrane glycoprotein encoded by the *endoglin* gene located on chromosome 9q34. This protein is composed of two constitutively phosphorylated subunits of 95 kDa each, forming a 180 kDa homodimeric mature protein [54]. CD105 is an accessory protein of the TGFβ complex. Upon activation of the TGFβ complex, the binding of endoglin results in the activation of Smad proteins leading to the regulation of various cellular processes such as cell proliferation, migration, differentiation, and angiogenesis [55]. Endoglin is predominantly expressed in endothelial cells where it is activated by hypoxia and TGFβ stimulation, whereas it is decreased by tumor necrosis factor α (TNFα) [56].

Interestingly, in breast, prostate, and gastric cancer, CD105 was found in endothelial cells forming immature tumor vasculature. In ccRCC, a subpopulation of cells representing <10% of the tumor mass showed CD105 upregulation. $CD105^+$ cells isolated by magnetic sorting displayed potent capability to grow as spheres and initiate tumors and metastases recapitulating the clear cell histological pattern in mice [48,57]. These cells also expressed mesenchymal markers CD44, CD90, CD29, CD73, and Vimentin; embryonic stem cell markers Oct3/4, Nanog and Nestin, and the embryonic renal marker Pax2 [48]. However, they did not express CD133, also known as human tubular progenitor cell marker [58]. $CD105^+$ CSCs were able to differentiate into epithelial and endothelial cells and generate $CD105^-$ cells. Additionally, immunohistochemical analysis of tumoural CD105 was found to correlate positively with nuclear grade and tumor stage, whereas endothelial expression correlated negatively with clinicopathological features [59]. Thus, CD105 has been proposed as the main marker for CSC identification in RCC.

CSCs have been found to secrete higher amount of exosomes and CSC-derived exosomes have been found involved in promoting angiogenesis in xenograft mice with renal cancer [57], metastatic niche formation in lung carcinoma [60] as well as invasion, migration and tumor growth in many other tumor types [61–65]. Interestingly, $CD105^+$ CSCs can release microvesicles and exosomes containing pro-angiogenic mRNAs (VEGF, FGF, MMP2, and 9) that trigger angiogenesis and promote the formation of a premetastatic niche *in vivo* [57]. Extracellular vesicles (EVs) derived from renal CSCs impaired T cell activation and dendritic cell differentiation by HLA-G promoting escape from the immune system [66].

Nevertheless, the use of CD105 as a renal CSCs marker has been questioned in many studies, where $CD105^-$ cells also showed CSC-like features [36].

CD133

Prominin-1 (CD133) is a transmembrane glycoprotein of 865 amino acids (120 kDa) encoded by the gene *PROM1* on chromosome 4p15 [67]. This protein exists in different isoforms and its regulation is complex [68,69]. Expressed by almost all cell types, CD133 localises in the plasma membrane suggesting its involvement in membrane remodeling and signal transduction [68]. Phosphorylation of CD133 results in the activation of PI3K/AKT signalling pathway [70,71]. Hypoxia, mTOR inhibition and TGFβ1 increased CD133 expression in lung cancer, pancreatic cancer, and hepatocellular carcinoma (HCC). Oct4 and Sox2 have been found to bind to the promoter region of CD133 inducing its activation in lung cancer cell lines. Along with its expression in

Table 1. Summary of putative CSC markers

Sample	Assay	Putative marker of the study	Positive markers	Negative markers	CSC features	Reference
769P	Side population		ABCB1	ABCC1, ABCG2	Clonogenic, tumourigenicity, resistance to chemo and radiotherapy	Huang *et al* [35]
786O	Sphere formation assay		CD73		tumourigenicity, resistance to radiotherapy	Song *et al* [36]
786O	Flow cytometry	Rh123			Spheroids in soft agar, proliferation, differentiation, tumourigenicity, resistance to radiotherapy	Lu *et al* [37]
786O, 769P, A704, Caki1, Caki2	Flow cytometry	USP21	ALDH		Sphere formation, clonogenic, proliferation, invasion	Peng *et al* [38]
ACHN	Side population		ALDH1	CD105, CD133	Sphere formation, self-renewal, tumourigenicity	Ueda *et al* [39]
ACHN, Caki1	Sphere formation assay		Oct4, Nanog, LIN28, KL4, Zeb1, Zeb2, N-cadherin, Vimentin, CD44, CD24	miR17	Sphere formation, self-renewal, differentiation, tumourigenicity	Lichner *et al* [40]
ACHN, Caki1	Flow cytometry	CD105	CD105, Oct4, Nanog, CD90, CD73	CD24, CD34, CD11, CD19, CD45	Spheroids in soft agar, hanging drops	Khan *et al* [41]
ACHN, Caki1	MACS		$CD133^{+}/CD24^{+}$, Oct4, Notch1, Notch2, Jagged1, Jagged2, DLL1, DLL 4		Self-renewal, invasion and migration, tumourigenicity, resistance to chemotherapy (sorafenib and cisplatin)	Xiao *et al* [42]
ACHN, Caki1, SMKTR2, SMKTR3, RenCa	Side population		DNAJB8		Tumourigenicity	Nishizawa *et al* [43]
ACHN, Caki2	Flow cytometry	ALDH1	Oct4, Nanog, Pax2		Self-renewal, clonogenic, tumourigenicity	
Caki1, Caki2, 786O, 769P	Sphere formation assay		CXCR4		Sphere formation, tumourigenicity	Micucci *et al* [44]
HEK293T	Sphere formation assay		$ALDH^{+}$, CD44, β-catenin, Notch1, Survivin, Vimentin, N-cadherin, Zeb1, Snail, Slug	CD24	Sphere formation, resistance to radiotherapy	Debeb *et al* [45]
RCC xenograft	Sphere formation assay		CD133/CXCR4		Sphere formation, tumourigenicity, resistance to chemotherapy	Varna *et al* [46]
RCC26, RCC53	Flow cytometry	CXCR4	CXCR4, CD24, CD29, CD44, CD73, Nanog, Oct4, Sox2	CD90, CD105, CD133, CXCR1, Vimentin, β-catenin	Sphere formation, tumourigenicity, resistance to chemotherapy	Gassenmeier *et al* [47]
RCCs	Flow cytometry	CD105	CD105, CD44, CD90, CD73, CD29, Nanog, Oct4, Vimentin, Nestin	CD133	sphere formation, clonogenic, differentiation, tumourigenicity	Bussolati *et al* [48]
RCCs	Flow cytometry	$CD133^{+}/CD34^{-}$	CD73, CD44, CD29, Vimentin		Nontumourigenic	Bruno *et al* [49]
RCCs	Flow cytometry	$CD133^{+}/CD24^{+}$	CTR2, Nanog, Oct4, Sox2	CD105, CD90	Resistance to chemotherapy	Galleggiante *et al* [50]
RCCs	Side population		CD133		Spheroids in soft agar, differentiation	Addla *et al* [51]
RenCa			DNAJB8		Side population, sphere formation, tumourigenicity	Yamashita *et al* [52]
SK-RC-42	Sphere formation assay		Oct4, Nanog, BMI, β-catenin	MHC-II, CD80	Sphere formation, tumourigenicity, resistance to radio and chemotherapy	Zhong *et al* [53]

stem and progenitor cells within normal tissues, CD133 has been proposed as a putative CSC marker across different tumor types [68].

$CD133^+$ cancer cells were able to form spheres, gave rise to tumors *in vivo* and exhibited chemoresistance properties in colorectal carcinoma (CRC), HCC, lung cancer, glioblastoma, pancreatic cancer, and ovarian cancer. On the contrary, sorted $CD133^+$ cells from RCC patients did not show tumourigenic capability *in vivo* although they expressed stem cell markers such as CD44, CD29, Vimentin, and Pax2 [58]. When co-transplanted with renal carcinoma cells, $CD133^+$ progenitors significantly enhanced tumor development and growth. The same result was obtain using $CD133^+$ cells derived from normal kidney tissue [72]. Of note, $CD105^+$ cells did not express CD133, suggesting that $CD133^+$ cells may represent renal resident adult progenitor cells rather than CSCs.

Interestingly, $CD133^+/CD24^+$ cells derived from RCC cell lines ACHN and Caki1 displayed sphere formation capability, enhanced invasion and migration properties, high colony formation efficiency in soft agar, and resistance to sorafenib and cisplatin [42].

Another interesting publication identified CD133 and CXCR4 co-expressing CSCs in spheres derived from RCC xenografts and tumor tissues. Increased expression of these markers was found in RCC patients after sunitinib treatment [46]. Nevertheless, whether the CD133 and CXCR4 positive or negative cells had detectable levels of CD105 was not assessed. Additionally, the gene expression profile as well as the tumourigenic potential of the spheres was not deciphered. Moreover, $CD133^-$ cells were also able to give rise to tumors in immunodeficient mice in glioblastoma and CRC [73,74].

Last, CD133 expression was found to correlate strongly with nuclear HIF1α in RCC patients [75,76]. CD133 mRNA levels in blood can be useful for identifying metastasis, predicting recurrence, and stratifying the patients into different risk groups for possible adjuvant treatment [77]. However, CD133 expression analysed by IHC in RCC patients was inconsistent and varied among different studies [55,78]. Because of the complex epigenetic and microenvironmental modulation together with higher protein processing and post-translation modifications, the applicability of CD133 as a CSC marker is limited [68].

CD44

CD44 is a transmembrane glycoprotein of 85 kDa (742 aa) encoded by the *CD44* gene located on chromosome 11. CD44 exists in more than 20 isoforms due to RNA alternative splicing, giving rise to different proteins in different cancer tissue subtypes. Due to the wide variety of isoforms, CD44 is involved in diverse biological processes such as cell–cell interaction, cell adhesion, migration, proliferation, differentiation, and angiogenesis [79].

Although other extracellular matrix (ECM) components such as collagen, growth factors, and metalloproteinases can interact with CD44, the extracellular glycosaminoglycan hyaluronan (HA) represent its primary ligand [80]. Binding of CD44 to HA promotes multiple signalling pathways including activation of receptor tyrosine kinases (RTK), TGFβ, MAPK, PI3K/AKT supporting cell proliferation, survival, invasion, and ultimately homing of CSCs in many tumor types [79,81]. In addition, CD44 has been found to be involved in the regulation of stem cell features via the Wnt/β-catenin signalling pathway and protein kinase C (PKC) [82]. Because of its tight interaction with the ECM, CD44 plays an essential role in modulation of the CSC niche. CSCs can synthetise HA to attract tumor-associated macrophages (TAMs) in the CSC niche. On the other hand, stromal cells will produce growth factors that regulate stem cell activity [79]. Enhanced CD44 expression was observed in RCC cell lines after co-culture with macrophages. This effect was the result of activation of the NFκβ pathway by the TNFα derived from TAMs [83]. TNFα enhanced migration and invasion of ccRCC cells together with down-regulation of E-cadherin expression and up-regulation of matrix metalloproteinase 9 (MMP9) and CD44 expression [84]. Interestingly, spheres derived from HEK293T, ACHN, Caki-1, and 786O renal cancer cell lines as well as $CD105^+$ cells isolated from RCC specimens showed the presence of a $CD44^+$ population with self-renewal properties, sphere formation capability and resistance to therapy [37,40,45]. Moreover, CD44 expression was found to be associated with Fuhrman grade, primary tumor stage, histological subtype, and poor prognosis in RCC patients [55,84,85]. Therefore, CD44 expression may serve as a prognostic and predictive as well as potential CSC marker for RCC [78].

In view of the involvement of CD44 in enhancing stem cell features in cancer cells and mediating crosstalk with the TME, CD44-based therapeutic strategies have been developed [82]. Monoclonal antibodies against CD44 are now in clinical trial for patients affected by AML, whereas knockdown of CD44 has been shown to increase sensitivity to chemotherapy in cell cultures derived from HCC, lung, breast and pancreatic cancers [80,86].

CD24

CD24 is a small cell surface protein molecule composed of only 27 amino acids, resulting in a molecular weight ranging between 20 and 70 kDa depending on the glycosylation status. It is encoded by the *CD24* gene located in the chromosome 6q21. CD24 is expressed in a wide variety of cell types, including hematopoietic cells [87]. Nevertheless, it is preferentially expressed in progenitor and stem cells. CD24 was shown to be an important maker for cancer diagnosis and prognosis in breast, non-small cell lung, colon, ovarian, and prostate cancer [87–89]. CD24 upregulation has been also found associated with CSCs and CSC features in many solid tumors. On the contrary, breast CSCs showed low CD24 levels, suggesting that the role of CD24 in stem cells may be tissue dependent [90–92]. Interestingly, high CD24 expression was observed in CSCs derived from the RCC cell line Caki2 [93], although contrasting results were reported when analysing the expression of CD24 together with the CSC marker CD44. Nevertheless, CD24 expression was found to correlate with tumor grade, overall survival, and disease-free survival in RCCs suggesting its prognostic significance [88]. Lazzeri *et al* identified a subpopulation of cells exhibiting self-renewal properties, expression of stem cell transcription factors, and the ability to regenerate kidney tissue upon injury. These cells derived from the human embryonic kidney expressed both CD24 and CD133 indicating they may represent putative normal kidney stem cells [94].

Because of the very limited research studies conducted on CD24 in RCC, we can conclude that, to date, no clear observation that CD24 can be used as a CSC marker in RCC has been made.

CXCR4

The CXC-chemokine receptor 4 (CXCR4 or CD184) is a seven transmembrane G protein-coupled receptor (GPCR) on the cell membrane. It is encoded by the *CXCR4* gene located on chromosome 2q22. CXCR4 selectively binds to the CXC chemokine stromal cell-derived factor 1 (SDF1 or CXCL12) leading to the activation of a variety of biological processes such as proliferation, survival, migration, stemness, and angiogenesis [95]. A number of signalling pathways are involved in the signal transduction. For instance, PLC/MAPK, PI3K/AKT, JAK/STAT, and the Ras/Raf pathway.

CXCR4 was found expressed in many different tumor tissues. It has been shown in breast, small cell lung cancer, neuroblastoma, and renal cancer that $CXCR4^+$ cells migrate towards tissues expressing high levels of SDF1 to metastasise [42,96,97]. Therefore, CXCR4/SDF1 is involved in cell-stroma interactions creating a permissive niche for metastasis [55]. Further, SDF1 stimulates adhesion of bone marrow progenitor/stem cells through CD44, demonstrating again a link between CD44 and CXCR4 signalling and TME [82].

Recent studies showed that $CXCR4^+$ cells derived from several RCC cell lines (RCC26 and RCC53; Caki1, Caki2, 786O, and 769P) express high levels of stem cell-associated genes and exhibit resistance to therapy (TKIs) and enhanced capability to form spheres *in vitro* and tumors *in vivo* compared to $CXCR4^-$ cells [44,47]. Conversely, inhibition of CXCR4 by ADM3100 or small interfering RNA (siRNA) impaired tumor formation [44,47]. Interestingly, loss of pVHL in ccRCCs as well as hypoxia led to increased CXCR4 and MMPs expression indicating HIF1α may be responsible for expansion of the CXCR4 population [98]. Supporting evidence showed that $CD133^+/CXCR4^+$ cells co-expressed HIF1α and were located in perinecrotic areas in RCCs [46]. Moreover, hypoxia promoted $CD133^+/CXCR4^+$ cells tumourigenicity whereas HIF2α was shown to be involved in the expansion of $CXCR4^+$ CSCs in four RCC cell lines [44]. The translational relevance of CXCR4 expression in the clinic was investigated in 2673 RCC patients by meta-analysis revealing a negative correlation between CXCR4 expression and overall survival (OS), cancer free survival, and disease free survival [99]. Taken together, these results indicate that CXCR4 may be explored as a potential CSC marker in RCC, perhaps in combination with a second marker. Nevertheless, care should be taken when choosing the appropriate marker for CSC isolation since too restrictive a selection may lead to failure in targeting all the stem-like cells present in the tumor population.

ALDH1

Aldehyde dehydrogenase 1 (ALDH1) is a cytosolic enzyme involved in the dehydrogenation of aldehydes to their corresponding carboxylic acids [100]. It is encoded by the *ALDH1* gene located on chromosome 9q21. ALDH1 plays an important role in cellular differentiation, proliferation, mobility, embryonic development, and organ homeostasis [39].

ALDH1 has been initially proposed and used as a marker to isolate stem cells from normal tissues such as brain and bone marrow with potential applications in the area of regenerative medicine [101,102]. More recently, the activity of cytosolic ALDH1 has also been shown to be a reliable marker of CSCs in several types of solid tumor, including breast, colon,

pancreas, lung, liver, prostate, and bladder [103,104]. Nevertheless, its prognostic significance in RCC is still unclear [105], although ALDH1 was found to correlate with tumor grade in RCC by Ozbek and co-authors [106].

High expression of ALDH1 was found in the side population (SP) derived from the RCC cell line ACHN compared to the non-SP. Analysis of the $ALDH1^+$ cells revealed enhanced sphere formation capability, self-renewal properties, tumourigenicity and high expression of stemness genes in the $ALDH1^+$ cells compared to $ALDH1^-$ cells. Moreover, drug treatment and hypoxic conditions were shown to increase the $ALDH1^+$ cell population *in vitro* [39].

Interestingly, a recent study investigated ALDH1 expression patterns in 24 types of normal human tissue as well as in primary epithelial tumor specimens and epithelial cancer cell lines, showing that ALDH1 may not be a suitable CSC marker for all tumor types especially in tissues where ALDH1 is constitutively highly expressed such as liver and pancreas [107]. Therefore, growing evidence suggests that ALDH1 is not only a putative stem cell marker, but may actually play multiple functional roles in regulating stem cell function [100].

ABCB5

The drug efflux transporter ABCB5 (ATP-binding cassette, sub-family B, member number 5), is an integral membrane glycoprotein encoded by the *ABCB5* gene located on chromosome 7p21. It is composed of 812 amino acids and has an overall molecular weight of 90 kDa. This protein is involved in the transport of small ions, sugar, peptides, and organic molecules across the plasma membrane against a concentration gradient by hydrolysis of ATP [108]. Because of its function, ABCB5 has been considered responsible for mediating therapeutic resistance [109].

ABCB5 has been found to be overexpressed in CSCs derived from melanoma, liver, and colorectal cancers. Moreover, it was found to be associated with tumor progression, chemotherapy resistance, and recurrence in many other tumor types [110]. For instance in renal cell cancer, *ABCB1* was found expressed in all cells and these tumors rarely respond to primary chemotherapy treatment [111]. Therefore, ABCB5 is exploited for distinguishing between stem cells (side population) and non-stem cells using flow cytometry.

Others

DNAJB8 is a member of the heat shock family of proteins (HSP40) that regulate chaperone activity. It is encoded by the *DNAJB8* gene located on chromosome 3q21. DNAJB8 is commonly expressed in the testis. Recently, Nishizawa *et al*, showed that DNAJB8 is expressed in different cancer cells including RCCs. In particular, the expression of DNAJB8 correlated with the SP compartment, and overexpression of the protein increased SP cells. Interestingly, DNAJB8 immunisation completely abolished tumor formation in mice, indicating that DNAJB8 can be a target for immunotherapy [43].

MicroRNAs (miRNAs) are non-coding small RNA molecules (22 nucleotides) involved in regulation of gene expression by translational repression, mRNA cleavage, and deadenylation. The role of miRNAs in CSCs has been described for different tumor types [112]. Six miRNAs involved in TGFβ and Wnt signalling pathways showed the most significant variations in expression by RT-PCR between spheres and parental cells derived from two metastatic RCC cell lines, ACHN and Caki1. Among those, miR17 was significantly downregulated in Caki1 and ACHN spheres. Inhibition of miR17 resulted in enhanced sphere formation indicating that TGFβ signalling plays an important role in renal CSCs and that miR17 impairs the signalling cascade by targeting the TGFβ signalling pathway [40].

Galleggiante *et al* isolated a subpopulation of cancer cells expressing CD133 and CD24 from 40 RCC samples. This population showed stem cell properties such as self-renewal, differentiation, tumourigenicity and expression of stemness-related transcription factors. $CD133^+/CD24^+$ cells appeared to be more undifferentiated compared to the corresponding tubular adult renal progenitor cells. Interestingly, these cells also expressed on the cell membrane the amino acid transporter CTR2 which was found to be involved in resistance to cisplatin [50].

Rhodamine 123 (Rh123) is a fluorescent dye that permeates the cell membrane and accumulates in the mitochondria proportionally to the mitochondrial membrane potential [113]. 786O cells were stained with Rh123 and sorted by flow cytometry into two population: $Rh123^{high}$ and $Rh123^{low}$. $Rh123^{high}$ exhibited high proliferative activity, differentiation, resistance to radiation, tumourigenic potential, and spheroid formation in soft agar, indicating Rh123 as an alternative method to isolate CSCs [37].

Finally, high CD73 expression was observed in spheres derived from the 786O RCC cell line. Moreover, $CD73^+$ cells displayed high levels of stemness-related transcription factors, resistance to radiotherapy, and tumourigenicity *in vivo* [36].

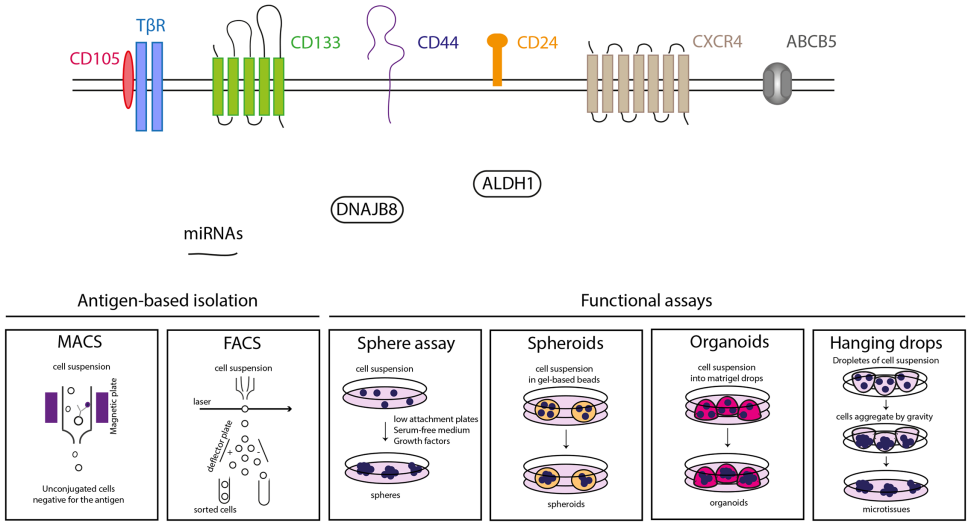

Figure 2. Identification and isolation of CSCs. Several potential CSC markers are shown. CD105, TβR, CD133, CD44, CD24, CXCR4, and ABCB5 are some of the most studied membrane CSC markers; whereas miRNAs, DNAJB8, ALDH1 stand out among the intracellular CSC markers. Based on these markers, FACS and MACS have been adopted as isolation methods for the separation of CSCs from other tumor cells. More recently, other techniques exploiting CSC properties have been developed with the aim of discovering potentially new biomarkers; these include the sphere assay, spheroid and organoid formation and hanging drops.

Isolation techniques

Different approaches for CSC isolation have been developed in recent years (Figure 2).

Antigen-based methods require labelling of the cells based on the expression of specific markers. These include magnetic bead-conjugated antibodies (MACS) [114,115], fluorescence-activated cell sorting (FACS) [116,117], and SP analysis [118,119]. Antigen selection often relies on markers which have been found relevant in developmental biology, embryonic, and hematopoietic stem cell studies. Nevertheless, dissociation of the tumor tissue into a single cell suspension may damage surface antigens limiting the efficiency of isolating CSCs using cellular marker-based methods [118]. Further, cells can lose viability upon enzyme treatment and after sorting procedures [120]. Cell sorting itself has proven to be imprecise with 1–3% of tumourigenic cells contaminating the non-tumourigenic population [19]. In addition, no generally applicable markers are known to date, and excessively permissive or restrictive labelling may have implications when developing therapeutic strategies targeting CSCs based on marker expression [19]. The identification and characterisation of putative CSC markers may also be achieved using functional assays [58]. In order to recreate the *in vivo* CSC niche using *in vitro* culture conditions, three-dimensional cell culture models were developed. Two different methods can be adopted for culturing CSCs in 3D: anchorage-independent and anchorage-dependent. While the anchorage-independent system takes advantage of the ability of CSCs to grow in suspension, the anchorage-dependent system uses scaffolds in order to enable cells to mimic their interaction with the ECM microenvironment and promote features of stemness. These methods are represented by spheroid [121,122] and organoid cultures [123–125], and the sphere formation assay [53,117,122] and hanging drops [126,127], respectively.

The ECM plays crucial roles in establishing the CSC niche and in mediating tumor drug resistance. It is composed of collagens, laminins, fibronectin, proteoglycans, and all the non-cellular components present in the tissues [128]. Different scaffolds can be used in these 3D CSC culture assays in order to mimic the ECM. Natural scaffolds include collagen, gelatin, elastin, fibrinogen, agarose, and alginate. Combinations of materials are also possible. Synthetic scaffolds can overcome the risk of contamination, degradation and batch-to-batch variations compared to natural scaffolds. These are mainly polymeric microparticles (e.g. hydrogels, PLGA, and PLC) [129].

Antigen-based methods

Magnetic bead-conjugated antibodies

MACS allows the isolation and enrichment of stem cells without further staining. Cells are labelled using antibodies conjugated to magnetic nanoparticles.

Labelled cells are then transferred into a column placed in a strong magnetic field. During this step, cells expressing the antigen will bind to the magnetic beads and stay in the column, whereas all the other cells that are negative for the antigen will flow through [114,115]. The population of interest can be subsequently eluted from the column.

Fluorescence-activated cell sorting

FACS is an alternative isolation method capable of sorting cells using fluorescently labelled antibodies targeting selected surface proteins or intracellular markers via direct or indirect immune fluorescence staining. Flow cytometry allows a sample of cells or particles in suspension to be separated through a narrow liquid stream. As the sample passes through a laser it allows for detection of size, granularity, and fluorescent properties of individual cells/particles [130]. Generally, FACS separation uses fluorochromes directly conjugated with either primary or secondary antibodies with different emission wavelengths. Although MACS is simpler and requires less complicated equipment than FACS, it is monoparametric and cannot isolate cells via multiple markers simultaneously [116,117].

Side population analysis

Hoechst SP analysis is one of several strategies used in stem cell studies [131]. SP is defined as a small fraction of cancer cells within a tumor exhibiting stem-like properties. The ability to discriminate the SP is based on the differential efflux of Hoechst 33342 by the multi-drug resistance ABC transporters [132]. CSCs possess higher activity and/or higher amounts of the ABC pumps, which are also responsible for the efflux of chemotherapeutic agents resulting in chemotherapy resistance of CSCs [109]. Therefore, SP stands out as the portion of cells able to extrude the dye against a concentration gradient when compared to cells not having stem cell features [119].

Identification of CSCs is achieved by specifically inhibiting ABC pumps using verapamil (100 μM) or reserpine (5 μM). Hoechst is excited at 405 nm and the blue signal is collected with a 450/40 nm bandpass filter, whereas the red fluorescence is collected with a 610/20 nm filter. Due to the high capability to extrude Hoechst dye, the side population can be defined as the population negative for Hoechst blue and Hoechst red [118]. Nevertheless, analysis of the SP has raised many concerns due to the dynamic nature of the dye efflux property as well as toxicity of the Hoechst dye making this technique highly variable [120,131].

SP isolation can also be achieved by using rhodamine 123 (Rh123) [37,133,134]. Rh123 is a mitochondrial dye that stains mitochondria with increasing intensity as cells become activated [37]. Rh123 fluorescence intensity is an index of mitochondrial mass, number and activation state, and multi-drug efflux pump activity [135]. Decreased intra-cellular accumulations of Rh123 results from the efflux of the dye.

Because non-stem cells may also express some of the ABC transporters, the isolation of CSCs through SP analysis is imperfect. While the SP may contain some non-stem cells, conversely some stem cells may not be located in the SP fraction [109].

Functional assays

Sphere formation assay

The sphere formation assay exploits the capability of CSCs to grow *in vitro* as spheroids in an anchorage-independent manner due to their mesenchymal phenotype. Cells are plated onto ultra-low attachment plates under serum-free medium conditions. Recent studies have demonstrated that CSC expansion requires medium lacking serum, which is believed to stimulate cellular differentiation [53,117,122]. The medium composition may vary but it is generally composed of DMEM/F12 medium supplemented with stem cell growth factors and/or hormones (i.e. bFGF, EGF, HGF, insulin, androgen, and progesterone). Therefore, cancer cells with stem cell-like features are able to proliferate and form spherical structures, whereas all the others will die. As a result of asymmetrical cell division, each CSC is capable of forming a sphere composed of both cells that have stem cell features as well as more differentiated cells. By passaging the spheres, CSCs can be enriched [53,117,122]. This results in an increased sphere number from one passage to the other. Compared to MACS and FACS, the sphere formation assay may retain clonal variations within the CSC pool by avoiding marker selection. Nevertheless, several critical parameters can impair CSC isolation and investigation using the sphere formation assay. These are: inappropriate seeding cell densities which can impact sphere formation and sphere clonality; the presence of quiescent CSCs which cannot be expanded using this method; and finally, possible overestimation of the stem cell frequency [136].

Hanging drops

In the hanging drop system, droplets of cell suspension are deposited onto a dish or into special plates (e.g. the GravityPLUS^{TM} Hanging Drop System,

InSphero AG, Switzerland and Perfecta3D® hanging drop plates, Sigma-Aldrich). Upon inversion of the tray, cells accumulate and aggregate by gravity on the surface of the liquid drop. This method is mainly used in the study of embryonic stem cells [126,127]. The Hanging Drop system allows efficient formation of uniform-size spheroids in a relative short time, making it a very useful tool for high-throughput screening studies.

Tumor spheroids

Incorporation of ECM proteins into serum-free medium may induce CSC-ECM interactions, normoxic/hypoxic conditions, metabolic gradients, and cooperation with stromal cell components through co-culture [129]. In the tumor spheroid model, a defined number of tumor cells are encapsulated into macro-beads derived from natural or synthetic scaffolds, and allowed to float into the medium until spheroids are formed. Another interesting version is based on plating tumor cells into soft agar [121,122]. An initial layer of agar composed of 0.6% agarose is deposited on the bottom of the plate. Once it has settled, a second layer of 0.3% agarose containing a tumor single cell suspension is placed onto the 0.6% agarose. An additional feeder layer of 0.3% agarose is then added. Agarose concentration may be adapted depending on the cancer type.

Tumor organoids

Organoids are formed by distributing dissociated tumor cells into Matrigel drops. Matrigel is a gel-based natural compound that consists of laminin, collagen IV, and enactin. Matrigel drops containing tumor cells are dispersed into normal tissue culture plates, and the cell-matrix mixture is incubated at 37 °C before adding the medium. Different ratios of cell suspension/Matrigel can be used depending on tumor type. Organoid medium is composed of adD-MEM/F12 supplemented with stem cell growth factors promoting Wnt signalling pathway (B27 supplement, N-acetyl-L-cysteine, EGF, A-83, Noggin, and R-spondin 1)[123–125].

Transplantation assay

To finally evaluate the tumourigenic potential of a tumor cell population expressing CSC features, cancer cells are serially transplanted into immunocompromised mice (serial tumor transplantations) at low cell density (limiting dilution assay). Cancer cells capable of developing tumors repeatedly, with recapitulation of the histological features and heterogeneity of the parental tumor, are defined as TICs [13]. The terms TIC and CSC are often used interchangeably, although TIC more specifically refers to the cell-of-origin. Nevertheless, the capability of a cancer cell to form tumors *in vivo* and to recapitulate the tumor heterogeneity of the corresponding parental tumor is one of the most known features of a CSC. Cancer cells capable of growing as 3D cultures but that do not have tumourigenic potential cannot be considered CSCs. Importantly, immune-deficient mice are not completely devoid of an immune system, and reactions involving host cytokines and immune cells may still take place when CSCs are transplanted into immunocompromised mice [19,137,138]. Additionally, cells need to adapt to the mouse milieu shaping CSC survival and properties. Therefore, optimisation of the transplantation assay as well as critical interpretation of the results should be adopted when studying CSC properties *in vivo*.

Lineage tracing

To determine the cell(s) of origin of cancer, normal cells are labelled under cell-specific promoters followed by induction of genetic modifications. In this way, a single cell or a population of cells is marked and their signature is transmitted to all the progeny [139]. Investigation of the cellular source of a tumor can be achieved by identifying and tracing over time these transformed cells responsible for forming the tumor. In parallel, this technique can be adopted to resolve the cell fate of tumor subpopulations in established tumors or to determine how cells behave in the context of the intact tissue or organism [13]. More importantly, genetic lineage tracing allows *in vivo* visualisation of stem cells [129].

Normal kidney stem cells

At the top of the hierarchy of cellular organisation, normal adult stem cells maintain tissue homeostasis and facilitate regeneration [140]. The kidneys carry out many different functions in the human body, including secreting hormones, absorbing minerals, filtering blood, and producing urine. Hence, impaired kidney function can ultimately lead to life threatening complications [141]. Tissue homeostasis in the kidney is limited and further diminished by age or disease [142]. This results in 20 million people worldwide suffering for chronic kidney disease (CKD) [143]. Therefore, identifying stem cell populations in the fetal and adult kidney is important for developing effective therapeutic applications and understanding stem cell biology within kidney tissue.

Here, we briefly describe the application of embryonic and adult renal stem cell markers with the aim to translate this knowledge to CSC biomarker discovery.

Wilms' tumor (WT) has proved to be the best model system for studying embryonic renal stem cells. These tumors result from differentiation arrest of embryonic progenitor cells committed to the nephric lineage [144]. Comparative gene expression profiling of WT and fetal human kidneys showed high concordance in the expression of the following markers: Pax2, Six1/2, NCAM, Fzd7, and Fzd2 [145,146]. Nevertheless, embryonic renal stem cells are entirely exhausted during nephrogenesis and the expression of these genes is rapidly lost during differentiation [147], limiting their utility to uncover purely embryonic-specific renal stem cell markers.

Adult renal stem cells have been investigated using BrdU staining [148]. BrdU is incorporated into dividing cells during the pulse phase, but further cell divisions will quickly dilute the stain leaving only cells that divide infrequently such as stem cells labelled with BrdU [149]. Nevertheless, some limitations must be considered such as adult stem cells dividing infrequently and not being labelled and, on the other hand, kidney has normally a limited mitotic index resulting in impaired signal dilution.

Following BrdU staining, a subset of cells expressing CD133 and CD24 were isolated from the urinary pole of Bowman's capsule and from the proximal tubules, in particular in the S3 segment. These cells also expressed stemness markers (i.e. Sox2, CD44, Oct4, and Vimentin), and could be discriminated by differential CD106 expression [150,151]. Stem cells derived from the Bowman's capsule were shown to move and differentiate from the urinary pole to the vascular pole acquiring podocyte traits (PDX marker) and losing stem cell markers (CD133 and CD24), whereas stem cells from the distal end of the proximal tubules were able to migrate within this segment [150–152]. The renal papilla offers a perfect niche for stem cells due to its hypoxic and hyperosmotic microenvironment [153]. Interestingly, Nestin and CD133 have been found to be expressed in stem cells derived from papillae [154].

Several studies have demonstrated that resident adult kidney stem cells are not the major player involved in tissue repair in the proximal tubule [155]. While differentiated cells undergo EMT and proliferate to repopulate the damaged area, some investigators have proposed that other non-resident stem cells such as bone marrow-derived stem cells or MSCs may be also involved in the process [147]. Nevertheless, these cells are defined as renal progenitor cells rather than renal stem cells due to their limited differentiation capability and lack of self-renewal properties.

Conclusions

Tumor relapse and metastasis are the primary causes of poor survival in ccRCC patients. CSCs are thought to be responsible for tumor propagation, metastasis formation, and treatment failure in many solid tumors, including renal cancer [25–34]. According to the CSC hypothesis, conventional therapies (i.e. radiation and chemotherapy) usually eliminate the majority of cells present in the tumor bulk while sparing the CSC pool [18]. This results in tumor recurrence. Therefore, understanding the mechanisms underlying metastasis and drug-resistance associated with CSCs may help to identify new therapeutic options.

Various approaches have been developed with the aim to successfully isolate and characterise CSCs, leading to the identification of a variety of CSC markers [156]. However, contrasting results have been reported in the literature on the use of these biomarkers [36,73,74,107]. Several studies have shown that CSC markers are not unique across tumor types; therefore, knowledge on relevant markers for NSCs or CSCs from other tumor types may not be useful in renal cancer. Growing evidence suggests that distinct CSC subpopulations may coexist within a heterogeneous tumor and new CSC (sub-)clones can be generated, selected and compete with each other similarly to the stochastic model during tumor progression and treatment, resulting in greater intra- and inter-CSC clone variability [140]. Therefore, some biomarkers can be relevant and applicable in certain phases during tumor development and progression, whereas they become obsolete in others.

Many scientists have raised concerns about the stem cell hypothesis. In particular, the fact that CSCs are considered as a rare slow-cycling subpopulation of cells questioned the possibility of their involvement in treatment failure, in support of mechanisms of acquired or intrinsic resistance [19]. Many studies demonstrate a higher CSC content than would be expected under the hypothesis of CSCs being a small subset, which may be explained by inefficient isolation methods affecting functional assays as well as xenograft rates [120]. Finally, if CSCs are slowly proliferating one could argue that CSCs are lost during *in vitro* manipulation, whereas these cells remain a constant fraction of the total population [109]. All

these concerns finds their explanation in the plastic nature of CSCs as well as technical issues.

CSC traits are sustained by interaction with the TME (niche) [21]. The CSC niche is an anatomically distinct TME present within a tumor that supports and sustains CSC properties [157,158]. It is composed of ECM, cancer-associated fibroblasts, mesenchymal stem cells, endothelial, and immune cells [137]. Stem cell niches are often localised in hypoxic regions where low O_2 levels induce slow cycle proliferation and minimise DNA damage due to ROS [44,46]. Processes such as inflammation, hypoxia, angiogenesis and EMT taking place within the TME contribute to the maintenance of CSC fate by acting on the most known pathways regulating CSCs: Wnt, SHH, Notch, TGFβ, and growth factor-receptor tyrosine kinase (RTK) [118,137,158,159]. Interestingly, tumor cells in the non-CSC compartment can spontaneously undergo EMT and acquire a CSC-like phenotype and surface marker expression [160]. At the same time, CSCs display different stemness features depending on the microenvironment, and these features may be transient [138,157]. The entire process has to be considered reversible, plastic, and dynamic.

Therefore, understanding the mechanisms underlying the properties of CSCs, and the integration of genomic and functional assays exploiting such features, may advance CSC studies as well as promote the identification of new biomarkers for renal CSCs. CSC assays should take into consideration the contribution of the niche. Moreover, optimisation of the transplantation assay using highly immune-deficient mice humanised with human TME and growth factors together with complementary lineage tracing analysis is of outmost importance for advancing CSC studies [137]. Last, combination of therapies specifically targeting CSCs by not only acting on CSC surface markers but also inhibiting CSC-related signalling pathways, delivering CSC-specific therapeutics as well as targeting the CSC niche together with conventional chemotherapy and radiotherapy, may ultimately lead to improved RCC patient survival [55,137].

Author contributions statement

C.C. conceived and designed the manuscript. C.C. and H.M. acquired and interpreted literature data. C.C. wrote the manuscript, whereas H.M. assisted in the manuscript drafting and revision.

References

1. Moch H. *Kidey Cancer* (World Cancer Report 2014 edn). International Agency for Research onCancer/World Health Organization: Lyon, 2014.
2. Bhatt JR, Finelli A. Landmarks in the diagnosis and treatment of renal cell carcinoma. *Nat Rev Urol* 2014; **11:** 517–525.
3. Moch H. An overview of renal cell cancer: pathology and genetics. *Semin Cancer Biol* 2013; **23:** 3–9.
4. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. IARC Press: Lyon, 2004.
5. Campbell SC, Rini BI. Renal cell carcinoma: clinical management. Springer: New York, 2013.
6. Frew IJ, Moch H. A clearer view of the molecular complexity of clear cell renal cell carcinoma. *Annu Rev Pathol* 2015; **10:** 263–289.
7. Messer J, Drabick J, Kaag M. Rational therapy for renal cell carcinoma based on its genetic targets. *Adv Exp Med Biol* 2013; **779:** 291–308.
8. Vikram R, Ng CS, Tamboli P, *et al*. Papillary renal cell carcinoma: radiologic-pathologic correlation and spectrum of disease. *Radiographics* 2009; **29:** 741–754; discussion 755–747.
9. Delahunt B, Eble JN, McCredie MRE, *et al*. Morphologic typing of papillary renal cell carcinoma: comparison of growth kinetics and patient survival in 66 cases. *Hum Pathol* 2001; **32:** 590–595.
10. Speicher MR, Schoell B, Du Manoir S, *et al*. Specific loss of chromosomes 1, 2, 6, 10, 13, 17, and 21 in chromophobe renal cell carcinomas revealed by comparative genomic hybridization. *Am J Pathol* 1994; **145:** 356–364.
11. Moch H, Montironi R, Lopez-Beltran A, *et al*. Oncotargets in different renal cancer subtypes. *Curr Drug Targets* 2015; **16:** 125–135.
12. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65:** 5–29.
13. Rycaj K, Tang DG. Cell-of-origin of cancer versus cancer stem cells: assays and interpretations. *Cancer Res* 2015; **75:** 4003–4011.
14. Gerstung M, Beisel C, Rechsteiner M, *et al*. Reliable detection of subclonal single-nucleotide variants in tumour cell populations. *Nat Commun* 2012; **3:** 811.
15. Ashkenazi R, Gentry SN, Jackson TL. Pathways to tumorigenesis–modeling mutation acquisition in stem cells and their progeny. *Neoplasia* 2008; **10:** 1170–1182.
16. Shackleton M, Quintana E, Fearon ER, *et al*. Heterogeneity in cancer: cancer stem cells versus clonal evolution. *Cell* 2009; **138:** 822–829.
17. Kreso A, Dick JE. Evolution of the cancer stem cell model. *Cell Stem Cell* 2014; **14:** 275–291.
18. Nguyen LV, Vanner R, Dirks P, *et al*. Cancer stem cells: an evolving concept. *Nat Rev Cancer* 2012; **12:** 133–143.
19. Kern SE, Shibata D. The fuzzy math of solid tumor stem cells: a perspective. *Cancer Res* 2007; **67:** 8985–8988.
20. Prasetyanti PR, Medema JP. Intra-tumor heterogeneity from a cancer stem cell perspective. *Mol Cancer* 2017; **16:** 41.
21. Atay S, Godwin AK. Tumor-derived exosomes: a message delivery system for tumor progression. *Commun Integr Biol* 2014; **7:** e28231.

22. Visvader JE. Cells of origin in cancer. *Nature* 2011; **469:** 314–322.
23. Lapidot T, Sirard C, Vormoor J, *et al*. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994; **367:** 645–648.
24. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997; **3:** 730–737.
25. Collins AT, Berry PA, Hyde C, *et al*. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005; **65:** 10946–10951.
26. Fang D, Nguyen TK, Leishear K, *et al*. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005; **65:** 9328–9337.
27. Hermann PC, Bhaskar S, Cioffi M, Heeschen C. Cancer stem cells in solid tumors. *Semin Cancer Biol* 2010; **20:** 77–84.
28. Kim CFB, Jackson EL, Woolfenden AE, *et al*. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005; **121:** 823–835.
29. Ma S, Chan K-W, Hu L, *et al*. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology* 2007; **132:** 2542–2556.
30. O'Brien CA, Pollett A, Gallinger S, *et al*. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007; **445:** 106–110.
31. Ricci-Vitiani L, Lombardi DG, Pilozzi E, *et al*. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007; **445:** 111–115.
32. Schatton T, Murphy GF, Frank NY, *et al*. Identification of cells initiating human melanomas. *Nature* 2008; **451:** 345–349.
33. Singh SK, Hawkins C, Clarke ID, *et al*. Identification of human brain tumour initiating cells. *Nature* 2004; **432:** 396–401.
34. Bussolati B, Dekel B, Azzarone B, *et al*. Human renal cancer stem cells. *Cancer Lett* 2013; **338:** 141–146.
35. Huang B, Huang YJ, Yao ZJ, *et al*. Cancer stem cell-like side population cells in clear cell renal cell carcinoma cell line 769P. *PLoS One* 2013; **8:** e68293.
36. Song L, Ye W, Cui Y, *et al*. Ecto-5'-nucleotidase (CD73) is a biomarker for clear cell renal carcinoma stem-like cells. *Oncotarget* 2017; **18**: 31977–31992.
37. Lu J, Cui Y, Zhu J, *et al*. Biological characteristics of Rh123high stem-like cells in a side population of 786-O renal carcinoma cells. *Oncol Lett* 2013; **5:** 1903–1908.
38. Peng L, Hu YI, Chen D, *et al*. Ubiquitin specific peptidase 21 regulates interleukin-8 expression, stem-cell like property of human renal cell carcinoma. *Oncotarget* 2016; **7:** 42007–42016.
39. Ueda K, Ogasawara S, Akiba J, *et al*. Aldehyde dehydrogenase 1 identifies cells with cancer stem cell-like properties in a human renal cell carcinoma cell line. *PLoS One* 2013; **8:** e75463.
40. Lichner Z, Saleh C, Subramaniam V, *et al*. miR-17 inhibition enhances the formation of kidney cancer spheres with stem cell/tumor initiating cell properties. *Oncotarget* 2015; **6:** 5567–5581.
41. Khan MI, Czarnecka AM, Lewicki S, *et al*. Comparative gene expression profiling of primary and metastatic renal cell carcinoma stem cell-like cancer cells. *PLoS One* 2016; **11:** e0165718.
42. Xiao W, Gao Z, Duan Y, *et al*. Notch signaling plays a crucial role in cancer stem-like cells maintaining stemness and mediating chemotaxis in renal cell carcinoma. *J Exp Clin Cancer Res* 2017; **36:** 41.
43. Nishizawa S, Hirohashi Y, Torigoe T, *et al*. HSP DNAJB8 controls tumor-initiating ability in renal cancer stem-like cells. *Cancer Res* 2012; **72:** 2844–2854.
44. Micucci C, Matacchione G, Valli D, *et al*. HIF2alpha is involved in the expansion of CXCR4-positive cancer stem-like cells in renal cell carcinoma. *Br J Cancer* 2015; **113:** 1178–1185.
45. Debeb BG, Zhang X, Krishnamurthy S, *et al*. Characterizing cancer cells with cancer stem cell-like features in 293T human embryonic kidney cells. *Mol Cancer* 2010; **9:** 180.
46. Varna M, Gapihan G, Feugeas J-P, *et al*. Stem cells increase in numbers in perinecrotic areas in human renal cancer. *Clin Cancer Res* 2015; **21:** 916–924.
47. Gassenmaier M, Chen D, Buchner A, *et al*. CXC chemokine receptor 4 is essential for maintenance of renal cell carcinoma-initiating cells and predicts metastasis. *Stem Cells* 2013; **31:** 1467–1476.
48. Bussolati B, Bruno S, Grange C, *et al*. Identification of a tumor-initiating stem cell population in human renal carcinomas. *FASEB J* 2008; **22:** 3696–3705.
49. Bruno S, Bussolati B, Grange C, *et al*. CD133+ renal progenitor cells contribute to tumor angiogenesis. *Am J Pathol* 2006; **169:** 2223–2235.
50. Galleggiante V, Rutigliano M, Sallustio F, *et al*. CTR2 identifies a population of cancer cells with stem cell-like features in patients with clear cell renal cell carcinoma. *J Urol* 2014; **192:** 1831–1841.
51. Addla SK, Brown MD, Hart CA, *et al*. Characterization of the Hoechst 33342 side population from normal and malignant human renal epithelial cells. *Am J Physiol Renal Physiol* 2008; **295:** F680–F687.
52. Yamashita M, Hirohashi Y, Torigoe T, *et al*. Dnajb8, a member of the heat shock protein 40 family has a role in the tumor initiation and resistance to docetaxel but is dispensable for stress response. *PLoS One* 2016; **11:** e0146501.
53. Zhong Y, Guan K, Guo S, *et al*. Spheres derived from the human SK-RC-42 renal cell carcinoma cell line are enriched in cancer stem cells. *Cancer Lett* 2010; **299:** 150–160.
54. Dallas NA, Samuel S, Xia L, *et al*. Endoglin (CD105): a marker of tumor vasculature and potential target for therapy. *Clin Cancer Res* 2008; **14:** 1931–1937.
55. Peired AJ, Sisti A, Romagnani P. Mesenchymal stem cell-based therapy for kidney disease: a review of clinical evidence. *Stem Cells Int* 2016; **2016:** 4798639.
56. Fonsatti E, Maio M. Highlights on endoglin (CD105): from basic findings towards clinical applications in human cancer. *J Transl Med* 2004; **2:** 18.
57. Grange C, Tapparo M, Collino F, *et al*. Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. *Cancer Res* 2011; **71:** 5346–5356.
58. Myszczyszyn A, Czarnecka AM, Matak D, *et al*. The role of hypoxia and cancer stem cells in renal cell carcinoma pathogenesis. *Stem Cell Rev* 2015; **11:** 919–943.
59. Saroufim A, Messai Y, Hasmim M, *et al*. Tumoral CD105 is a novel independent prognostic marker for prognosis in clear-cell renal cell carcinoma. *Br J Cancer* 2014; **110:** 1778–1784.
60. Jung T, Castellana D, Klingbeil P, *et al*. CD44v6 dependence of premetastatic niche preparation by exosomes. *Neoplasia* 2009; **11:** 1093–1105.

61. Hu Y, Yan C, Mu L, *et al*. Fibroblast-derived exosomes contribute to chemoresistance through priming cancer stem cells in colorectal cancer. *PLoS One* 2015; **10:** e0125625.
62. Kumar D, Gupta D, Shankar S, *et al*. Biomolecular characterization of exosomes released from cancer stem cells: possible implications for biomarker and treatment of cancer. *Oncotarget* 2015; **6:** 3280–3291.
63. Bourkoula E, Mangoni D, Ius T, *et al*. Glioma-associated stem cells: a novel class of tumor-supporting cells able to predict prognosis of human low-grade gliomas. *Stem Cells* 2014; **32:** 1239–1253.
64. Wang M, Zhao C, Shi H, *et al*. Deregulated microRNAs in gastric cancer tissue-derived mesenchymal stem cells: novel biomarkers and a mechanism for gastric cancer. *Br J Cancer* 2014; **110:** 1199–1210.
65. Lin R, Wang S, Zhao RC. Exosomes from human adipose-derived mesenchymal stem cells promote migration through Wnt signaling pathway in a breast cancer cell model. *Mol Cell Biochem* 2013; **383:** 13–20.
66. Grange C, Tapparo M, Tritta S, *et al*. Role of HLA-G and extracellular vesicles in renal cancer stem cell-induced inhibition of dendritic cell differentiation. *BMC Cancer* 2015; **15:** 1009.
67. Li Z. CD133: a stem cell biomarker and beyond. *Exp Hematol Oncol* 2013; **2:** 17.
68. Grosse-Gehling P, Fargeas CA, Dittfeld C, *et al*. CD133 as a biomarker for putative cancer stem cells in solid tumours: limitations, problems and challenges. *J Pathol* 2013; **229:** 355–378.
69. Zhong LY, Du X, Geng SX, *et al*. [Expression of CD133 in the bone marrow of patients with myelodysplastic syndrome and its clinical significance]. *Nan Fang Yi Ke Da Xue Xue Bao* 2011; **31:** 854–855.
70. Sahlberg SH, Spiegelberg D, Glimelius B, *et al*. Evaluation of cancer stem cell markers CD133, CD44, CD24: association with AKT isoforms and radiation resistance in colon cancer cells. *PLoS One* 2014; **9:** e94621.
71. Park EK, Lee JC, Park JW, *et al*. Transcriptional repression of cancer stem cell marker CD133 by tumor suppressor p53. *Cell Death Dis* 2015; **6:** e1964.
72. Bussolati B, Bruno S, Grange C, *et al*. Isolation of renal progenitor cells from adult human kidney. *Am J Pathol* 2005; **166:** 545–555.
73. Shmelkov SV, Butler JM, Hooper AT, *et al*. CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. *J Clin Invest* 2008; **118:** 2111–2120.
74. Beier D, Hau P, Proescholdt M, *et al*. CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. *Cancer Res* 2007; **67:** 4010–4015.
75. Sun C, Song H, Zhang H, *et al*. CD133 expression in renal cell carcinoma (RCC) is correlated with nuclear hypoxia-inducing factor 1alpha (HIF-1alpha). *J Cancer Res Clin Oncol* 2012; **138:** 1619–1624.
76. Maeda K, Ding Q, Yoshimitsu M, *et al*. CD133 modulate HIF-1alpha expression under hypoxia in EMT phenotype pancreatic cancer stem-like cells. *Int J Mol Sci* 2016; **17:** 1025.
77. Feng G, Jiang F, Pan C, *et al*. Quantification of peripheral blood CD133 mRNA in identifying metastasis and in predicting recurrence of patients with clear cell renal cell carcinoma. *Urol Oncol* 2014; **32:** 44.e49–14.
78. Zhang Y, Sun B, Zhao X, *et al*. Clinical significances and prognostic value of cancer stem-like cells markers and vasculogenic mimicry in renal cell carcinoma. *J Surg Oncol* 2013; **108:** 414–419.
79. Basakran NS. CD44 as a potential diagnostic tumor marker. *Saudi Med J* 2015; **36:** 273–279.
80. Thapa R, Wilson GD. The importance of CD44 as a stem cell biomarker and therapeutic target in cancer. *Stem Cells Int* 2016; **2016:** 2087204.
81. Ponta H, Sherman L, Herrlich PA. CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 2003; **4:** 33–45.
82. Zoller M. CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? *Nat Rev Cancer* 2011; **11:** 254–267.
83. Ma C, Komohara Y, Ohnishi K, *et al*. Infiltration of tumor-associated macrophages is involved in CD44 expression in clear cell renal cell carcinoma. *Cancer Sci* 2016; **107:** 700–707.
84. Mikami S, Mizuno R, Kosaka T, *et al*. Expression of TNF-alpha and CD44 is implicated in poor prognosis, cancer cell invasion, metastasis and resistance to the sunitinib treatment in clear cell renal cell carcinomas. *Int J Cancer* 2015; **136:** 1504–1514.
85. Li X, Ma X, Chen L, *et al*. Prognostic value of CD44 expression in renal cell carcinoma: a systematic review and meta-analysis. *Sci Rep* 2015; **5:** 13157.
86. Jin L, Hope KJ, Zhai Q, *et al*. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. *Nat Med* 2006; **12:** 1167–1174.
87. Fang X, Zheng P, Tang J, *et al*. CD24: from A to Z. *Cell Mol Immunol* 2010; **7:** 100–103.
88. Arik D, Can C, Dündar E, *et al*. Prognostic significance of CD24 in clear cell renal cell carcinoma. *Pathol Oncol Res* 2017; **23:** 409–416.
89. Yun E-J, Zhou J, Lin C-J, *et al*. Targeting cancer stem cells in castration-resistant prostate cancer. *Clin Cancer Res* 2016; **22:** 670–679.
90. Ricardo S, Vieira AF, Gerhard R, *et al*. Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. *J Clin Pathol* 2011; **64:** 937–946.
91. Gao M-Q, Choi Y-P, Kang S, *et al*. CD24+ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. *Oncogene* 2010; **29:** 2672–2680.
92. Honeth G, Bendahl P-O, Ringnér M, *et al*. The CD44+/CD24- phenotype is enriched in basal-like breast tumors. *Breast Cancer Res* 2008; **10:** R53.
93. Jaggupilli A, Elkord E. Significance of CD44 and CD24 as cancer stem cell markers: an enduring ambiguity. *Clin Dev Immunol* 2012; **2012:** 708036.
94. Lazzeri E, Crescioli C, Ronconi E, *et al*. Regenerative potential of embryonic renal multipotent progenitors in acute renal failure. *J Am Soc Nephrol* 2007; **18:** 3128–3138.
95. Busillo JM, Benovic JL. Regulation of CXCR4 signaling. *Biochim Biophys Acta* 2007; **1768:** 952–963.
96. Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. *Semin Cancer Biol* 2004; **14:** 171–179.

97. Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer* 2004; **4:** 540–550.
98. Struckmann K, Mertz K, Steu S, *et al*. pVHL co-ordinately regulates CXCR4/CXCL12 and MMP2/MMP9 expression in human clear-cell renal cell carcinoma. *J Pathol* 2008; **214:** 464–471.
99. Cheng B, Yang G, Jiang R, *et al*. Cancer stem cell markers predict a poor prognosis in renal cell carcinoma: a meta-analysis. *Oncotarget* 2016; **7:** 65862–65875.
100. Ma I, Allan AL. The role of human aldehyde dehydrogenase in normal and cancer stem cells. *Stem Cell Rev* 2011; **7:** 292–306.
101. Kastan MB, Schlaffer E, Russo JE, *et al*. Direct demonstration of elevated aldehyde dehydrogenase in human hematopoietic progenitor cells. *Blood* 1990; **75:** 1947–1950.
102. Corti S, Locatelli F, Papadimitriou D, *et al*. Identification of a primitive brain-derived neural stem cell population based on aldehyde dehydrogenase activity. *Stem Cells* 2006; **24:** 975–985.
103. Marcato P, Dean CA, Pan D, *et al*. Aldehyde dehydrogenase activity of breast cancer stem cells is primarily due to isoform ALDH1A3 and its expression is predictive of metastasis. *Stem Cells* 2011; **29:** 32–45.
104. Resetkova E, Reis-Filho JS, Jain RK, *et al*. Prognostic impact of ALDH1 in breast cancer: a story of stem cells and tumor microenvironment. *Breast Cancer Res Treat* 2010; **123:** 97–108.
105. Abourbih S, Sircar K, Tanguay S, *et al*. Aldehyde dehydrogenase 1 expression in primary and metastatic renal cell carcinoma: an immunohistochemistry study. *World J Surg Oncol* 2013; **11:** 298.
106. Ozbek E, Calik G, Otunctemur A, *et al*. Stem cell markers aldehyde dehydrogenase type 1 and nestin expressions in renal cell cancer. *Arch Ital Urol Androl* 2012; **84:** 7–11.
107. Deng S, Yang X, Lassus H, *et al*. Distinct expression levels and patterns of stem cell marker, aldehyde dehydrogenase isoform 1 (ALDH1), in human epithelial cancers. *PLoS One* 2010; **5:** e10277.
108. Chen KG, Szakács G, Annereau J-P, *et al*. Principal expression of two mRNA isoforms (ABCB 5alpha and ABCB 5beta) of the ATP-binding cassette transporter gene ABCB 5 in melanoma cells and melanocytes. *Pigment Cell Res* 2005; **18:** 102–112.
109. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 2005; **5:** 275–284.
110. Wilson BJ, Saab KR, Ma J, *et al*. ABCB5 maintains melanoma-initiating cells through a proinflammatory cytokine signaling circuit. *Cancer Res* 2014; **74:** 4196–4207.
111. Dean M. ABC transporters, drug resistance, and cancer stem cells. *J Mammary Gland Biol Neoplasia* 2009; **14:** 3–9.
112. Liu C, Tang DG. MicroRNA regulation of cancer stem cells. *Cancer Res* 2011; **71:** 5950–5954.
113. Baracca A, Sgarbi G, Solaini G, *et al*. Rhodamine 123 as a probe of mitochondrial membrane potential: evaluation of proton flux through F(0) during ATP synthesis. *Biochim Biophys Acta* 2003; **1606:** 137–146.
114. Miltenyi S, Müller W, Weichel W, *et al*. High gradient magnetic cell separation with MACS. *Cytometry* 1990; **11:** 231–238.
115. Hu P, Zhang W, Xin H, *et al*. Single cell isolation and analysis. *Front Cell Dev Biol* 2016; **4:** 116.
116. Moghbeli M, Moghbeli F, Forghanifard MM, *et al*. Cancer stem cell detection and isolation. *Med Oncol* 2014; **31:** 69.
117. Khan MI, Czarnecka AM, Helbrecht I, *et al*. Current approaches in identification and isolation of human renal cell carcinoma cancer stem cells. *Stem Cell Res Ther* 2015; **6:** 178.
118. Abbaszadegan MR, Bagheri V, Razavi MS, *et al*. Isolation, identification, and characterization of cancer stem cells: a review. *J Cell Physiol* 2017; **232:** 2008–2018.
119. Goodell MA, Stem cell identification and sorting using the Hoechst 33342 side population (SP). *Curr Protoc Cytom* 2005; **Chapter 9**: Unit9.18.
120. Hill RP. Identifying cancer stem cells in solid tumors: case not proven. *Cancer Res* 2006; **66:** 1891–1895; discussion 1890.
121. Yuhas JM, Li AP, Martinez AO, *et al*. A simplified method for production and growth of multicellular tumor spheroids. *Cancer Res* 1977; **37:** 3639–3643.
122. Weiswald LB, Bellet D, Dangles-Marie V. Spherical cancer models in tumor biology. *Neoplasia* 2015; **17:** 1–15.
123. Clevers H. Modeling development and disease with organoids. *Cell* 2016; **165:** 1586–1597.
124. Drost J, Karthaus WR, Gao D, *et al*. Organoid culture systems for prostate epithelial and cancer tissue. *Nat Protoc* 2016; **11:** 347–358.
125. Pauli C, Hopkins BD, Prandi D, *et al*. Personalized in vitro and in vivo cancer models to guide precision medicine. *Cancer Discov* 2017; **7:** 462–477.
126. Kurosawa H. Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. *J Biosci Bioeng* 2007; **103:** 389–398.
127. Thoma CR, Zimmermann M, Agarkova I, *et al*. 3D cell culture systems modeling tumor growth determinants in cancer target discovery. *Adv Drug Deliv Rev* 2014; **69–70:** 29–41.
128. Egeblad M, Rasch MG, Weaver VM. Dynamic interplay between the collagen scaffold and tumor evolution. *Curr Opin Cell Biol* 2010; **22:** 697–706.
129. Bielecka ZF, Maliszewska-Olejniczak K, Safir IJ, *et al*. Three-dimensional cell culture model utilization in cancer stem cell research. *Biol Rev Camb Philos Soc* 2017; **92:** 1505–1520.
130. Kalisky T, Quake SR. Single-cell genomics. *Nat Methods* 2011; **8:** 311–314.
131. Golebiewska A, Brons NHC, Bjerkvig R, *et al*. Critical appraisal of the side population assay in stem cell and cancer stem cell research. *Cell Stem Cell* 2011; **8:** 136–147.
132. Salcido CD, Larochelle A, Taylor BJ, *et al*. Molecular characterisation of side population cells with cancer stem cell-like characteristics in small-cell lung cancer. *Br J Cancer* 2010; **102:** 1636–1644.
133. Greve B, Kelsch R, Spaniol K, *et al*. Flow cytometry in cancer stem cell analysis and separation. *Cytometry A* 2012; **81:** 284–293.
134. Vieyra DS, Rosen A, Goodell MA. Identification and characterization of side population cells in embryonic stem cell cultures. *Stem Cells Dev* 2009; **18:** 1155–1166.
135. Bertoncello I, Williams B. Hematopoietic stem cell characterization by Hoechst 33342 and rhodamine 123 staining. *Methods Mol Biol* 2004; **263:** 181–200.
136. Pastrana E, Silva-Vargas V, Doetsch F. Eyes wide open: a critical review of sphere-formation as an assay for stem cells. *Cell Stem Cell* 2011; **8:** 486–498.
137. Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? *Cell Stem Cell* 2015; **16:** 225–238.
138. Oskarsson T, Batlle E, Massague J. Metastatic stem cells: sources, niches, and vital pathways. *Cell Stem Cell* 2014; **14:** 306–321.

139. Kretzschmar K, Watt FM. Lineage tracing. *Cell* 2012; **148:** 33–45.
140. Baccelli I, Trumpp A. The evolving concept of cancer and metastasis stem cells. *J Cell Biol* 2012; **198:** 281–293.
141. Rosenberg ME, Gupta S. Stem cells and the kidney: where do we go from here? *J Am Soc Nephrol* 2007; **18:** 3018–3020.
142. Pleniceanu O, Harari-Steinberg O, Dekel B. Concise review: kidney stem/progenitor cells: differentiate, sort out, or reprogram? *Stem Cells* 2010; **28:** 1649–1660.
143. Breyer MD, Susztak K. The next generation of therapeutics for chronic kidney disease. *Nat Rev Drug Discov* 2016; **15:** 568–588.
144. Moch H, Humphrey PA, Ulbright TM, *et al*. WHO Classification of Tumors of the Urinary System and Male Genital Organs. 2016; **8:** 400.
145. Dekel B, Metsuyanim S, Schmidt-Ott KM, *et al*. Multiple imprinted and stemness genes provide a link between normal and tumor progenitor cells of the developing human kidney. *Cancer Res* 2006; **66:** 6040–6049.
146. Pode-Shakked N, Metsuyanim S, Rom-Gross E, *et al*. Developmental tumourigenesis: NCAM as a putative marker for the malignant renal stem/progenitor cell population. *J Cell Mol Med* 2009; **13:** 1792–1808.
147. Bussolati B, Camussi G. Therapeutic use of human renal progenitor cells for kidney regeneration. *Nat Rev Nephrol* 2015; **11:** 695–706.
148. Humphreys BD. Cutting to the chase: taking the pulse of label-retaining cells in kidney. *Am J Physiol Renal Physiol* 2015; **308:** F29–F30.
149. Huling J, Yoo JJ. Comparing adult renal stem cell identification, characterization and applications. *J Biomed Sci* 2017; **24:** 32.
150. Ronconi E, Sagrinati C, Angelotti ML, *et al*. Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol* 2009; **20:** 322–332.
151. Sagrinati C, Netti GS, Mazzinghi B, *et al*. Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol* 2006; **17:** 2443–2456.
152. Angelotti ML, Ronconi E, Ballerini L, *et al*. Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury. *Stem Cells* 2012; **30:** 1714–1725.
153. Oliver JA, Klinakis A, Cheema FH, *et al*. Proliferation and migration of label-retaining cells of the kidney papilla. *J Am Soc Nephrol* 2009; **20:** 2315–2327.
154. Oliver JA, Sampogna RV, Jalal S, *et al*. A subpopulation of label-retaining cells of the kidney papilla regenerates injured kidney medullary tubules. *Stem Cell Rep* 2016; **6:** 757–771.
155. Humphreys BD. Kidney injury, stem cells and regeneration. *Curr Opin Nephrol Hypertens* 2014; **23:** 25–31.
156. Peired AJ, Sisti A, Romagnani P. Renal cancer stem cells: characterization and targeted therapies. *Stem Cells Int* 2016; **2016:** 8342625.
157. Chaffer CL, Brueckmann I, Scheel C, *et al*. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc Natl Acad Sci U S A* 2011; **108:** 7950–7955.
158. Carnero A, Lleonart M. The hypoxic microenvironment: a determinant of cancer stem cell evolution. *Bioessays* 2016; **38** (Suppl 1): S65–S74.
159. Cabarcas SM, Mathews LA, Farrar WL. The cancer stem cell niche–there goes the neighborhood? *Int J Cancer* 2011; **129:** 2315–2327.
160. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol* 2017; **14:** 611–629.